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Diagnostic value of urinary enzyme determination in renal transplantation

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Abstract The measurement of enzyme activity in urine provides a sensitive assessment for renal tubular cell damage. The present study was undertaken to evaluate the clinical value of the determination of tubular brush-border-associated enzymes, alkaline phosphatase (AP), gamma-glutamyl transferase (GGT), leucine aminopeptidase (LAP), and dipeptidyl peptidase IV (DPP), of patients with normal graft function (NOR, $n = 20$), with acute tubular necrosis (ATN, $n = 11$), with an acute rejection episode (*ARE*, $n = 17$) after transplantation, and of healthy persons ($n = 20$). The second urine of the morning was collected daily during the patients' stay in hospital. The enzyme activities were measured at 25 °C and were expressed as U/mmol creatinine. The enzymuria in NOR is higher than in healthy controls, but is still in the normal range. By 5 days after transplantation the initial increased excretion declines as the graft function improves. Elevated enzymuria (DPP 0.69 ± 0.56, AP 3.06 ± 3.24, GGT 4.16 ± 4.13, and LAP 1.39 ± 1.27) was observed during the rejection episodes. Two days before clinical diagnosis of rejection, the release of DPP-IV and GGT increases to double, and the AP and LAP increases to 3 times the value on the fourth day before rejection. Successful treatment of rejection coincided with a quick return by the third day of the rejection period to the previous enzyme distribution. In ATN no decrease of enzymuria occurs and the excretion is much higher than in ARE. Our method with the every day monitoring of kidney graft function offers the possibility for the early diagnosis of acute rejection.

Key words Enzymuria · Dipeptidyl aminopeptidase IV · Alkaline phosphatase · Gamma-glutamyl transferase · Leucine aminopeptidase

Introduction

Nowadays renal transplantation provides a well-established treatment for patients with end-stage renal disease. On the basis of present evidence many immunological, inflammatory, and enzymatic abnormalities are noted after transplantation. Detection of these problems can be used for diagnostic and therapeutic purposes [1, 3, 4, 7]. Kidney transplants are frequently followed by rejection (35–40 %). The clinical signs can be only detected when relatively large deterioration had happened in the renal tissue [1, 2, 4, 6]. In the past decade, the evaluation of urinary enzymes has played a key role in the non-invasive diagnosis of rejection. Diagnostic significance of urinary enzymes increased due to adoption of the new, nephrotoxic immunsuppressive therapy, because the clinical signs of rejection became less characteristic and so the diagnosis become more difficult.

Deterioration of graft function can be caused by acute rejection episode(s) (ARE), acute tubular necro-

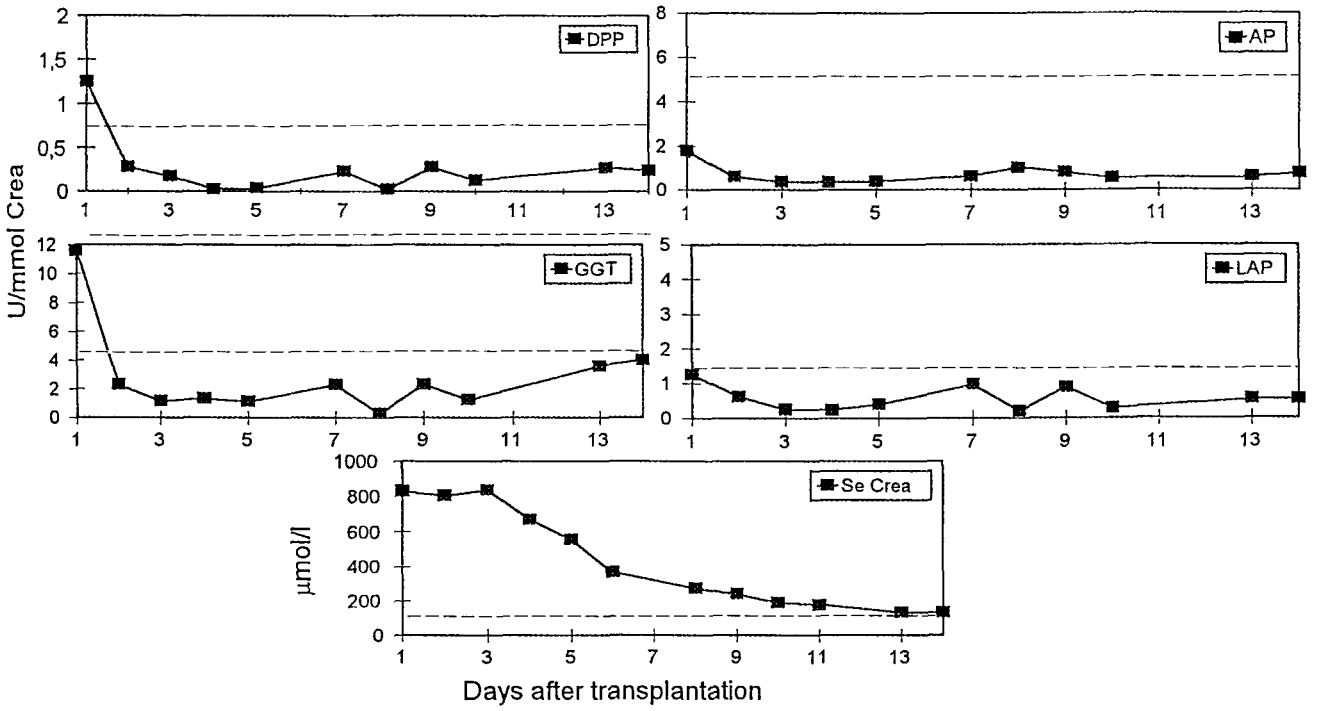

Fig. 1 Excretion patterns of brush border enzymes and serum creatinine concentration in an allograft recipient with normal graft function (*y-axis* shows the enzyme activities in U/mmol creatinine, *horizontal dotted lines* mark the upper reference limits of normal range)

sis (ATN), the cyclosporin (CyA)-induced nephrotoxicity, and surgical complications. It is difficult to make a prompt differential diagnosis because the symptoms are very similar in some cases. Our study was undertaken:

1. To find those enzymes which have a diagnostic and predictive value for rejection in the posttransplant period of renal transplant patients
2. To determine the dynamic changes of the different enzyme concentrations in the different complications (ARE, ATN) leading to deterioration of the graft function
3. To apply our results to the selection of the proper treatment and to monitor and promptly predict the usefulness of therapy (e.g. steroid "shot")
4. To create the enzyme diagnostic laboratory background for the clinicans to help them to discriminate between the different complications before the clinical symptoms occur, without expensive and dangerous invasive investigations.

The application of enzyme diagnostic methods in everyday practice would be beneficial from the point of view of the patients, because it can improve the chance of longer graft survival and this leads to a significant drop in the hospital costs.

Patients and methods

Healthy controls

The reference population (controls) consisted of 20 healthy persons (female = 11, male 9, age = 24–43 years) chosen from the staff and medical students of our clinic. They had no cardiac, liver, or renal disease, diabetes, or hypertension, and had normal blood cell counts, normal urine analysis, and normal findings in serum urea and creatinine. They were not on any medication for a week before the time of sampling. The female volunteers were neither pregnant nor menstruating at the time of sample collection.

Renal transplant patients

Samples from 54 renal transplant patients were collected and evaluated. Patients were on our standard immunsuppressive protocol of cyclosporin A (CyA) and IV methylprednisolone. After admission the patients were classified into three groups according to the diagnosis of the clinicians:

1. Patients ($n = 17$) with ARE were described as having fever (mostly in the mornings), rising serum creatinine, diminished daily urine volume, graft tenderness and enlargement, and increased resistance index. When there was a strong clinical suspicion of rejection antirejection therapy was started.
2. Patients ($n = 11$) with ATN were characterized by high serum creatinine, greatly diminished daily urine volume or no urine at all. These patients required dialysis following transplantation without any sign of other types of kidney diseases.
3. The uncomplicated group (NOR) of transplanted patients ($n = 20$) were classified as having an immediate onset of renal function and a spontaneous decrease in serum creatinine level immediately after transplantation.

Six investigated patients (OTHER) could not be classified into any group mentioned above. "Shot" therapy was applied for these

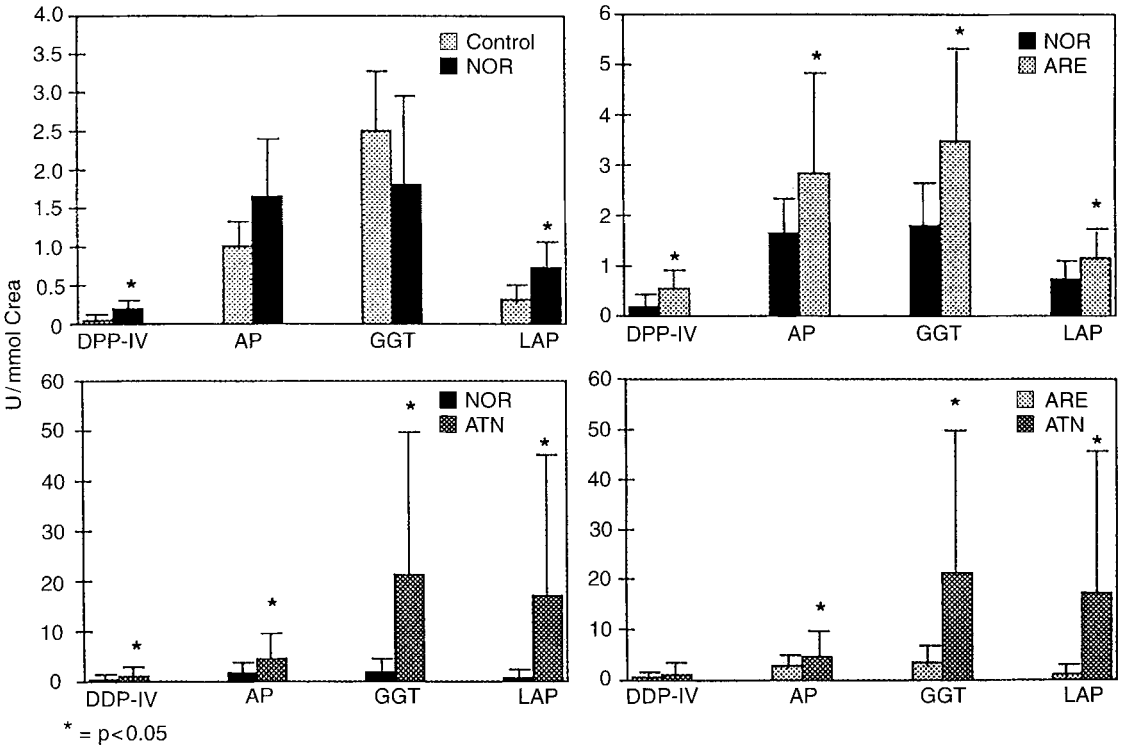

Fig. 2 The average enzyme activities are compared in the different groups; control vs patients with normal graft function (NOR), NOR vs patients with acute rejection episode (ARE), NOR vs patients with acute tubular necrosis (ATN), ARE vs ATN. (*y-axis* represents the enzyme activities in U/mmol creatinine, $P < 0.05$)

Table 1 Average enzymuria (U/mmol creatinine) of all investigated groups (*NOR* patients having immediate onset renal function posttransplantation, *ARE* patients having acute rejection episodes, *ATN* patients having acute tubular necrosis, *DPP-IV* dipeptidyl peptidase IV, *AP* alkaline phosphatase, *GGT* gamma-glutamyl transferase, *LAP* leucine aminopeptidase)

	DPP-IV	AP	GGT	LAP
Control	0.053	1.008	2.504	0.313
NOR	0.186	1.645	1.803	0.728
ARE	0.551	2.837	3.472	1.158
ATN	1.063	4.497	21.246	17.101
ARE/NOR (%)	352	178	222	185
ATN/NOR (%)	572	273	1180	2349
ATN/ARE (%)	162	159	612	148

patients, however, they had other problems but the symptoms were similar to rejection. They had no acute rejection episode diagnosed by clinicians retrospectively [5].

Collection of samples

The second midstream urines of the morning (2 ml) were collected from both control subjects and patients during the patients' stay in the hospital (2–3 weeks). The samples were centrifuged (2000 × *g* for 15 min), the creatinine concentration measured (Jaffe method-VP Super System, single channel, ABBOT Laboratories Diagnostic Division Texas, USA) and the supernatants were stored at 4 °C until the day of measurement.

Determination of enzyme activities

The determination of enzyme activities were measured kinetically on an ELISA reader (Anthos Reader 2001, Anthos Labtec Instrument) 405 nm, 25 °C, by monitoring the increase of absorbance due to the release of chromogenic (the 4-nitroanilin/or at the ALP: 4-nitrophenol) substrates. The activities were expressed as U/l. In order to account for variations due to urine concentration without collecting 24-hour specimens, urinary enzyme/creatinine ratios were calculated and expressed as U/mmol creatinine. This method compensates for varying rates of urinary flow. The reference intervals of these enzymes at 25 °C are: DPP-IV 0.13–1.08, AP 0.03–0.32, GGT: 0.8–4.01, and LAP: 0.1–0.61.

The onset of rejection was indicated by the first day of "shot" therapy. The 4-day period before and the 4–6 days following rejection were investigated Students' *T*-probe was applied for statistical analysis and the differences were significance if $P < 0.05$.

Results

The investigated enzymes have controversial diagnostic value in the 4–5 postoperative days, although they can indicate the ability of the graft to regenerate (Fig. 1). In the NOR group the significant enzymuria in the early postoperative period decreases to within the normal range by the fifth day (Fig. 1). The enzymuria, except

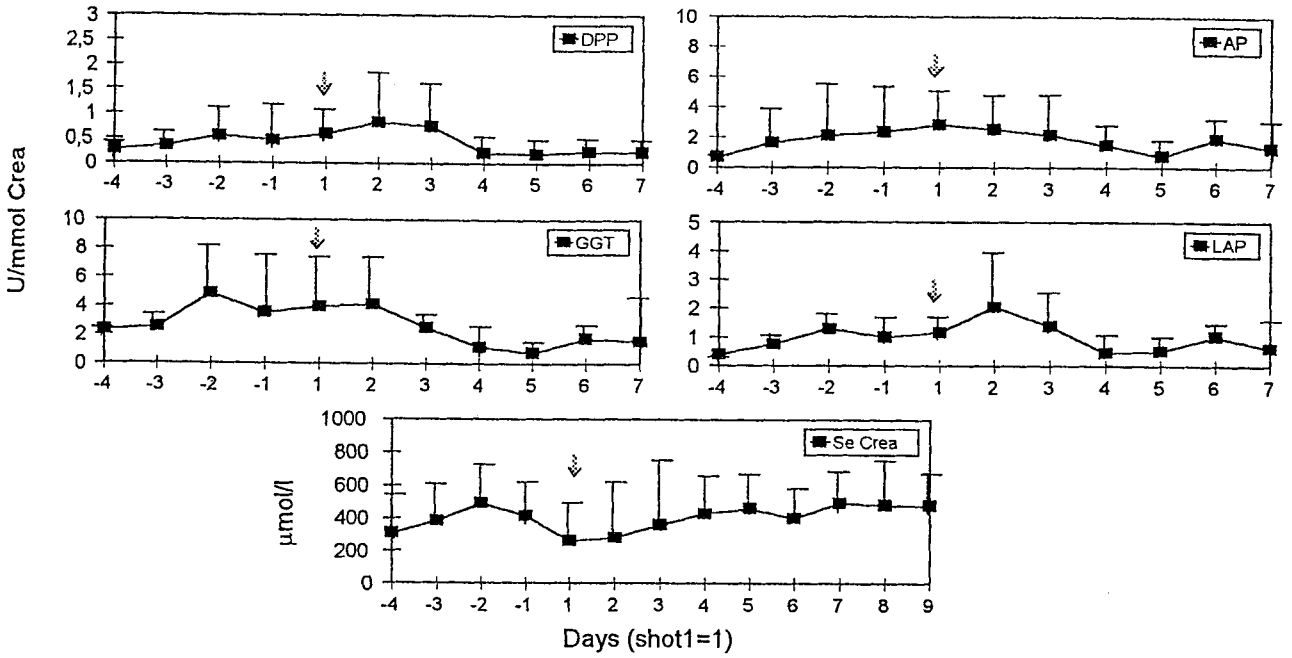

Fig. 3 Excretion patterns of brush border enzymes and serum creatinine concentration in patients with ARE before, during, and after "shot" therapy. (*y-axis* shows the enzyme activities in U/mmol creatinine, ↓ indicates the onset of rejection)

Table 2 Enzyme activities of ARE patients ($n = 17$) before during and after rejection episode. (Data are given as a % of the level 4 days before onset of rejection episode). "Shot" therapy was begun an day 1

Napok	DPP-IV (%)	AP (%)	GGT (%)	LAP (%)
-4	100	100	100	100
-3	126	239	107	190
-2	208	306	206	324
-1	172	343	151	251
1	221	408	168	292
2	308	364	174	514
3	282	310	107	345
4	77	213	50	112
5	72	111	33	131
6	91	272	75	257
7	89	186	67	155

for GGT, is slightly elevated when compared with the healthy controls (Fig. 2, Table 1). In the ATN group the prompt enzymuria does not decrease, it can even rise further (Fig. 2, Table 1). The measured and calculated enzymuria is several times higher than in the NOR ARE groups (Fig. 2, Table 1). In the ARE group, the immediate postoperative elevated enzymuria decreases similarly to the NOR group, then it starts to rise as a consequence of acute rejection. The enzymuria precedes the occurrance of clinical symptoms of acute rejection by 1–3 days (Figs. 2, 3, Table 1, 2). Two days before clinical diagnosis of rejection, the release of DPP-IV and GGT increases to double, and the AP and LAP increases to 3 times the value on the 4th day before rejection (Fig. 3, Table 2). The highest level of these enzymes was observed on the second day of the rejection period (Fig. 3, Table 2). On the third day of the rejection period the enzymuria decreases significantly showing the beneficial effect of "shot" therapy (Table 2). The enzyme activities of other group (OTHER) were under the reference limit before and during "shot" therapy.

Discussion

We evaluated the urinary enzyme activities to find those enzymes which have a diagnostic and predictive value for acute rejection in the early posttransplant period of renal transplant patients. Nowadays, applied immunsuppression therapy requires a new approach in the measurement of tubular brush border enzymes in the urine. The determination of urinary enzymes offers a good possibility for the diagnosis of different pathological alterations of renal grafts. Acute rejection episodes in kidney transplant patients with conventional immunsuppression therapy (azathioprine + prednisolone) were often predicted by an increasing enzymuria of certain brush border enzymes. The CyA therapy was followed by a more effective immunsuppression but also a higher degree of nephrotoxicity. Since then the clinical and laboratory diagnosis of acute rejection periods proved to be more difficult because as the frequency of acute re-

jection episodes decreased significantly, the clinical signs became less characteristic, and the nephrotoxicity meant a "noisy" background for the urinary enzyme "signals".

Acute rejection episodes are the most frequent postoperative complication of kidney transplantation so the early diagnosis and treatment is very important. The regular monitoring of these investigated urinary enzyme activities throughout the posttransplant period would be valuable in indicating graft function and in predicting effectiveness of the rejection treatment. Their simplicity, sensitivity, and non-invasive nature make the assay of urinary enzymes a powerful addition to the clinician's diagnostic armoury.

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